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Long-term changes in sublittoral macroalgal assemblages related to water quality improvement

Abstract: Sampling of sublittoral macroalgal assemblages was carried out in 1982 and 1999 along the coast of Catalonia (Northwestern Mediterranean, Spain) in order to study long-term changes in species composition. Ordination analysis revealed significant differences in species composition and abundance between the two study periods. The number of stations with indicators of eutrophication, such as species of *Chaetomorpha*, *Cladophora*, and *Ulva*, decreased from 1982 to 1999. This decrease was not balanced by an increase in species typical of pristine environments, such as large perennial brown algae (in particular, *Cystoseira mediterranea*), but rather by an increase of stress-resistant species such as *Corallina elongata*. The observed shifts seem to be driven by the decrease in nutrient loadings in the water column, resulting from the progressive implementation of sewage treatment management in the study region since the mid-1970s.

Keywords: cleanup treatment; environmental quality; long-term changes; macroalgal assemblages; Mediterranean Sea.

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Introduction

Benthic organisms tend to integrate the effects of long-term exposure to natural and anthropogenic disturbances (Borowitzka 1972). For this reason, studies of benthic community structure and composition may be a useful tool for assessing changes in water quality and environmental status (Fairweather 1990). In fact, analyses of rocky shore benthic assemblages have been used in quality assessments of coastal areas, and several monitoring methods have been described (Panayotidis et al. 2004, Ballesteros et al. 2007, Pinedo et al. 2007, Sfriso et al. 2007, Wells et al. 2007, Díez et al. 2009, Sfriso and Facca 2011).

The Mediterranean is almost devoid of kelp beds (Zabala and Ballesteros 1989), which characterize most shallow water sublittoral ecosystems in temperate seas. In the absence of disturbances, shallow sublittoral habitats in pristine Mediterranean rocky zones are usually covered by various species of brown algae, with members of the genus *Cystoseira* forming the most complex communities (Ros et al. 1985). In natural upper sublittoral environments in the Northwestern Mediterranean, macroalgal assemblages are dominated mainly by *Cystoseira mediterranea* or *Cystoseira amentacea* var. *stricta* (Ballesteros 1988, 1995, Sales and Ballesteros 2009). When the concentration of organic matter and nutrients in the water column increases, these canopy-forming algae are generally replaced by turfs dominated by *Corallina elongata* and mussels (*Mytilus galloprovincialis*) (Bellan-Santini 1965, 1968, Bellan and Bellan-Santini 1972, Ballesteros 1992, Giaccone 1993, Arévalo et al. 2007). Fast-growing ephemeral algae, such as green (*Chaetomorpha*, *Cladophora*, *Ulva*) and blue-green algae are generally dominant at the highest eutrophication levels (Golubic 1970, Bellan and Bellan-Santini 1972, Belsher 1974, 1979, Ballesteros et al. 1984, Cloern 2001, Arévalo et al. 2007). These changes in the dominance of macroalgal species in concert with nutrient levels make them potential bioindicators of ecological quality status, and macroalgal assemblages have been considered as a biological quality element in the frame of the European Water Framework Directive (2000/60/EC) (Panayotidis et al. 2004, Ballesteros et al. 2007).

The effects of pollution on macroalgal assemblages have been reported in previous studies (Bellan-Santini 1968, Munda 1974, Anger 1975, Littler and Murray 1975, Murray and Littler 1978, Belsher 1979, Kautsky et al. 1986, Hardy et al. 1993, Soltan et al. 2001), and several works have examined long-term changes following the onset of sewage discharge (Chapman et al. 1995, Gorostiaga and Díez 1996, Underwood and Chapman 1996, Díez et al. 1999, Bishop et al. 2002, Eriksson et al. 2002, Echevarri-Erasun et al. 2007). However, only a few studies have focused on the recovery of macroalgal assemblages following clean-up measures (Hardy et al. 1993, Bokn et al. 1996, Archambault et al. 2001, Díez et al. 2009), and only two have addressed Mediterranean communities (Soltan et al. 2001, Tsiamis et al. 2013).

Catalonia (Northwestern Mediterranean) is a densely populated region in Spain, and sections of its coast have been affected by high levels of urban and industrial development and coastal modification, although other sections are devoted to agriculture and tourism development (Pinedo et al. 2007). Coastal water quality has been extensively modified by sewage discharge coming from urban areas, industry, and agriculture (Arévalo et al. 2007, Ballesteros et al. 2007, Pinedo et al. 2007, Romero et al. 2007). However, in 1968, the Catalan Water Agency began to establish water treatment plants along the Catalan coast. As a result, a large proportion of raw wastewater discharge at the coast of northern Catalonia was intercepted and treated between 1974 and 1985, and this was extended from 1993 onward to the most populated and developed sector further south (Anonymous 1981, 1982, 1992, 2009) (Figure 1).

In the current study, we describe changes in the abundance of macroalgae in upper sublittoral assemblages between 1982 and 1999 following the establishment of treatment plants in Catalonia. We analyzed changes in species composition and abundance of the assemblages with multivariate methods, focusing on pollution-sensitive species, such as the brown alga *Cystoseira mediterranea*, and on the so-called thionitrophilic species, i.e., some green algae belonging to the Orders Ulvales and Cladophorales.

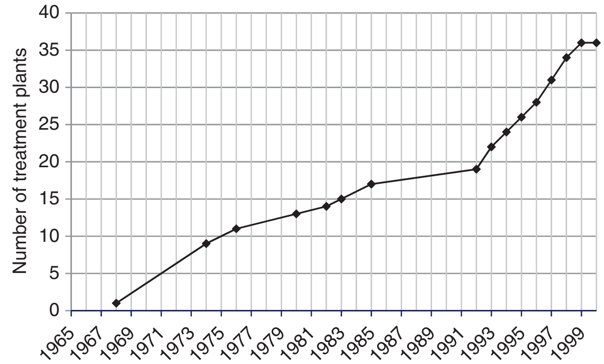

Figure 1 Cumulative number of treatment plants from the 1960s until 2000 along the coast of Catalonia.

Materials and methods

Study site and data collection

The Catalan coast extends for more than 400 km in northeastern Spain, along the western Mediterranean (Figure 2). Eighty-eight stations were sampled between May and June in 1982 and 1999, coinciding with maximal seasonal development of macroalgal rocky shore assemblages in the Mediterranean (Ballesteros 1989). Samples were consistently collected in the upper sublittoral zone, on gently sloping rocks exposed to surf and on both natural and artificial (i.e., external harbor structures) substrata. One sample of 225 cm² rocky surface was collected at every station using a hammer and chisel. This surface area is large enough to permit a quantitative description of the upper sublittoral macroalgal assemblages in the Northwestern Mediterranean (Coppejans 1980, Verlaque 1987, Ballesteros 1992). Samples were preserved in 4% formalin-seawater and sorted in the laboratory. Algae were identified to the lowest taxonomic level possible and quantified in terms of coverage. Species coverage was measured by spreading specimens over a laboratory tray to form a thin layer and estimating the horizontal surface area (cm²) covered (Ballesteros 1986, Sales et al. 2012).

Data analysis

The data set including all samples was analyzed using CANOCO software (ter Braak 1988). Species appearing in <2% of the samples were eliminated from the analysis. A detrended correspondence analysis (DCA) was performed

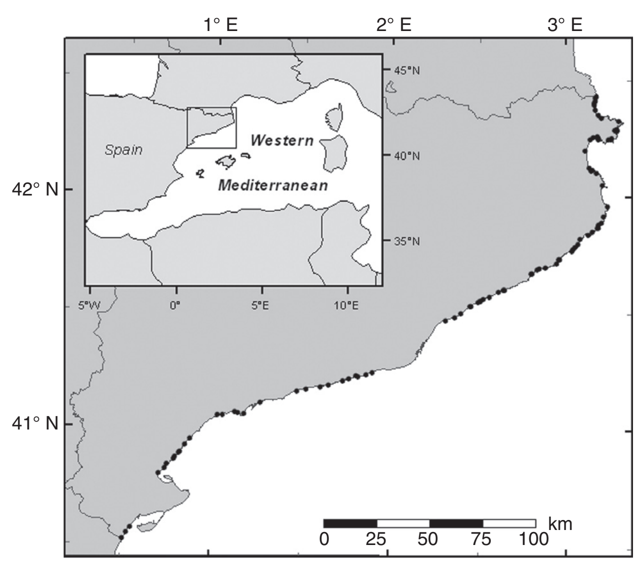

Figure 2 Location of study area (Catalonia, Northwestern Mediterranean). Dots represent sampling stations.

following the recommendations of ter Braak and Šmilauer (1998), indicating a unimodal response of species variance. DCA was carried out to register shifts in the macroalgal assemblage composition between 1982 and 1999. An additional analysis investigated changes over time for the most abundant species (the fucoids *Cystoseira mediterranea* and *Cystoseira compressa*, the red calcareous alga *Corallina elongata*, and the green alga *Ulva rigida*) and for the total coverage in the sampled sites. A multiway analysis of variance (MANOVAR) of the relative coverage values was used to test differences over time for each species. Absolute values were used for the total coverage. Data were ranked in order to meet the assumptions of normality and homogeneity. Univariate tests were performed to test the effect of each variable in the interaction between time and coverage values.

Results

Changes in temporal species composition

The ordination of the sampling stations, using DCA of coverage values collected in 1982 and 1999, reveals differences in species composition for several sampling stations between the two study periods (Figure 3A). The first and second axes explain 10.8% of the variance (6.2% and 4.6% for axis I and II, respectively). The ordination by species scores is illustrated in Figure 3B. Table 1 shows the species included in the ordination analysis. Samples are distributed along the first axis in Figure 3A, with those dominated by *Cystoseira mediterranea* on the right side of the plot and those dominated by green algae (*Ulva*, *Chaetomorpha*, and *Cladophora* species) on the left side (see Figure 3B). Samples from sites dominated by *Corallina elongata* are situated in between (Figure 3B), with values from 4 to 5 along the first axis. The segregation of samples along the second axis is based on the dominance of the different green algal species and seems to involve mainly samples from 1982 (Figure 3A).

The most striking change between 1982 and 1999 was the dramatic decline in the coverage and presence of green algae and other filamentous species. Samples collected in 1982 are clearly distributed along axes I and II, with the highest dispersion of the samples along the second axis (Figure 3A). On the other hand, samples collected in 1999 show a low dispersion along the second axis, and most of samples are situated along the first axis, mainly close to the positions of *C. mediterranea* and *C. elongata*. It is interesting to highlight the large shifts in the ordination plane of some study sites between 1982 and 1999 (Figure 3A). Several samples dominated by green algae in 1982 and situated at values between 1 and 4 on the first axis (Figure 3A) shift in 1999 to higher values along the first axis, with a dominance of *C. elongata* or *C. mediterranea* (Figure 3B). This shift to the right side of the ordination is not observed for station 95. In fact, this sample was dominated by *Dictyota dichotoma* and *C. elongata* with some *Ulva rigida* in 1982, but shifted to being dominated by *Cladophora coelothrix* and *U. rigida* in 1999, showing a displacement to the left in the ordination (Figure 3A).

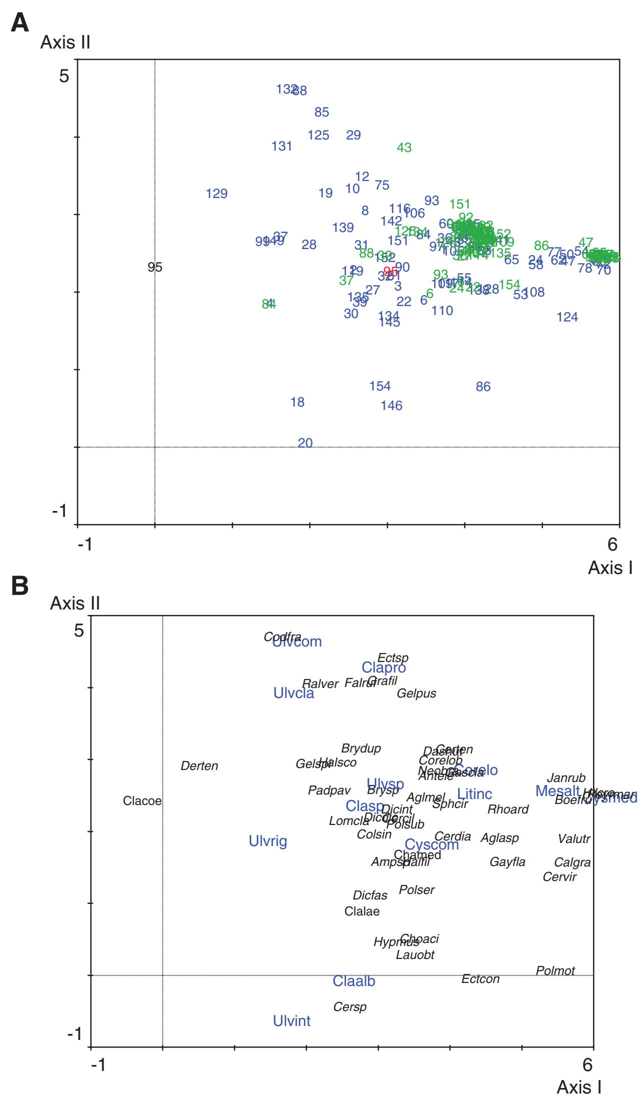

Figure 3 (A) DCA ordination plot showing the distribution of samples using species cover as variable in 1982 (blue) and 1999 (green). Station 95, which is discussed in the text, is colored in red in 1982 and in black in 1999. (B) DCA ordination plot showing the distribution of species using species cover as variable. For abbreviations of species names, see Table 1. Species in blue are discussed in the text.

Table 1 Species included in the ordination analysis (Figure 3): abbreviations, full names, and authorities.

Aglmel	*Aglaozonia melanoidea* (Schousboe ex Bornet) Sauvageau (stadium)	Ectsp	*Ectocarpus* sp.
Aglsp	*Aglaozonia* sp.	Falruf	*Falkenbergia rufolanosa* (Harvey) Schmitz (stadium)
Ampsp	*Amphiroa* sp.	Gayfla	Gayliella flaccida (Harvey ex Kützing) T.O. Cho *et* L.J. McIvor
Antele	*Antithamnionella elegans* (Berthold) Price *et* John	Gascla	*Gastroclonium clavatum* (Roth) Ardissone
Boefru	*Boergeseniella fruticulosa* (Wulfen) Kylin	Gelpus	*Gelidium* gr. *pusillum* (Stackhouse) Le Jolis
Brydup	*Bryopsis duplex* De Notaris	Gelspi	*Gelidium spinosum* (S.G. Gmelin) P.C. Silva
Brysp	*Bryopsis* sp.	Grafil	*Grateloupia filicina* (Lamouroux) C. Agardh
Calgra	*Callithamnion granulatum* (Ducluzeau) C. Agardh	Halfil	*Halopteris filicina* (Grateloup) Kützing
Cercil	*Ceramium ciliatum* (Ellis) Ducluzeau	Halsco	*Halopteris scoparia* (L.) Sauvageau
Cerdia	*Ceramium diaphanum* (Lightfoot) Roth	Hilcro	*Hildenbrandia crouaniorum* J. Agardh
Cersp	*Ceramium* sp.	Hypmus	*Hypnea musciformis* (Wulfen) Lamouroux
Certen	*Ceramium tenerrimum* (Martens) Okamura	Janrub	*Jania rubens* (L.) Lamouroux
Cerrub	*Ceramium virgatum* Roth	Lauobt	*Laurencia obtusa* (Hudson) Lamouroux
Chamed	*Chaetomorpha mediterranea* (Kützing) Kützing	Litincr	*Lithophyllum incrustans* Philippi
Choaci	*Chondracanthus acicularis* (Roth) Fredericq	Lomcla	*Lomentaria clavellosa* (Turner) Gaillon
Claalb	*Cladophora albida* (Nees) Kützing	Mesalt	*Mesophyllum alternans* (Foslie) Cabioch *et* Mendoza
Clacoe	*Cladophora coelothrix* Kützing	Neobra	*Neogoniolithon brassica-florida* (Harvey) Setchell *et* Mason
Clalae	*Cladophora laetevirens* (Dillwyn) Kützing	Padpav	*Padina pavonica* (L.) Thivy
Clapro	*Cladophora prolifera* (Roth) Kützing	Peyrmar	*Peyssonnelia rosa-marina* Boudouresque *et* Denizot
Clasp	*Cladophora* sp.	Polmot	*Polysiphonia mottei* Lauret
Codfra	*Codium fragile* (Suringar) Hariot	Polser	*Polysiphonia sertularioides* (Grateloup) J. Agardh
Colsin	*Colpomenia sinuosa* (Mertens ex Roth) Derbès *et* Solier	Polsub	*Polysiphonia subulata* (Ducluzeau) Crouan *et* Crouan
Corelo	*Corallina elongata* Ellis *et* Solander	Ralver	*Ralfsia verrucosa* Areschoug
Corelob	*Corallina elongata* (bases) Ellis *et* Solander (bases)	Rhoard	*Rhodymenia ardissonei* (Kuntze) Feldmann
Cyscom	*Cystoseira compressa* (Esper) Gerloff *et* Nizamuddin	Sphcir	*Sphacelaria cirrosa* (Roth) C. Agardh
Cysmed	*Cystoseira mediterranea* Sauvageau	Ulvcla	*Ulva clathrata* (Roth) C. Agardh
Dashut	*Dasya hutchinsiae* Harvey	Ulvcom	*Ulva compressa* Linnaeus
Derten	*Derbesia tenuissima* (Moris *et* De Notaris) Crouan *et* Crouan	Ulvint	*Ulva intestinalis* Linnaeus
Dicdic	*Dictyota dichotoma* (Hudson) Lamouroux	Ulvrig	*Ulva rigida* C. Agardh
Dicint	*Dictyota dichotoma* var. *intricata* (C. Agardh) Greville	Ulvsp	*Ulva* sp.
Dicfas	*Dictyota fasciola* (Roth) Lamouroux	Valutr	*Valonia utricularis* (Roth) C. Agardh
Ectcon	*Ectocarpus confervoides* (Dillwyn) Lyngbye		

Changes in species coverage

The number of macroalgal species recorded was 122 in 1982 and 79 in 1999. Only 47 taxa were common to both years. *Cystoseira mediterranea* and *Corallina elongata* were dominant both in 1982 and 1999, with total coverage values being 29% (1982) and 44% (1999) for *C. mediterranea* and 10% (1982) and 18% (1999) for *C. elongata*. Green algae were only significantly present during 1982, comprising 10% of the total coverage, of which *Ulva rigida* and *Ulva compressa* were the most abundant species. *Cystoseira compressa* and *Lithophyllum incrustans* were also abundant in 1982 (7% and 6%, respectively) and 1999 (4% and 11%, respectively).

In 1982, 16 species were required to make up 80% coverage, of which two (*Cystoseira caespitosa* and *U. compressa*) were absent in 1999. However, low coverages (<3%) were recorded for these two species in 1982. Conversely, five species accounted for more than 80% of total coverage in 1999, and four of the five species were also present in 1982; only *Mesophyllum alternans*, which contributed 4% of the total coverage in 1999, was not observed in 1982.

Multivariate test statistics showed that the interaction of time with tested species coverage was significant

($p<0.001$). Changes in the total coverage value and specific coverage values of *C. elongata*, *C. mediterranea*, *C. compressa*, and *U. rigida* contributed to the long-term changes in algal coverage. Univariate tests showed a significant increase in total coverage from 1982 to 1999 ($p<0.001$). *Corallina elongata* coverage was significantly higher in 1999 than in 1982 ($p<0.01$). However, coverage of the green alga *Ulva rigida* significantly decreased from 1982 to 1999 ($p<0.001$). Finally, neither *C. mediterranea* nor *C. compressa* showed significant differences in coverage between sampling years.

Temporal changes in both coverage and number of species of opportunistic green algae associated with eutrophication were observed: 13 species were found in 1982, but only 5 were observed in 1999 (Table 2).

Discussion

Dramatic changes in the macroalgal assemblages of the upper sublittoral were observed between 1982 and 1999, mainly involving a decrease in the number and abundance of green algae species (Table 2). *Cladophora albida* and *Cladophora prolifera* showed the largest decreases in coverage between 1982 and 1999. The majority of these species are indicative of eutrophication (Borowitzka 1972, López Gappa et al. 1993, Rodríguez-Prieto and Polo 1996, Arévalo et al. 2007, Piñón-Gimate et al. 2008, Teichberg et al. 2010) as the abundance of these species increases in the presence of excess nutrients. Moreover, a decrease in opportunistic green algae has usually been reported following decommissioning of outfalls (Smith et al. 1981, Archambault et al. 2001, Soltan et al. 2001, Tsiamis et al. 2013). The presence of these ephemeral green algae in 1982 was therefore likely to be related to the numerous sewage outfalls along the Catalonian coast and the resultant high nutrient loadings in inshore coastal waters. The reduction of green algal dominance at most sampling stations in 1999 suggests an improvement in coastal water quality linked to the major progress in sewage water treatment undertaken by the Government of Catalonia since the mid-1970s and particularly during the 1990s. Our results suggest that, before the introduction of extensive water treatment practices in Catalonia, sewage outfalls provided sufficient nutrients to enable green algae to dominate in many locations.

Table 2 Green algal species (orders Ulvales and Cladophorales) recorded in the study area (Catalonia, Northwestern Mediterranean) in 1982 and 1999.

	1982	1999
Blidingia minima (Nägeli ex Kützing) Kylin	+	
Chaetomorpha aerea (Dillwyn) Kützing	+	
Cladophora albida (Nees) Kützing	+	+
Cladophora prolifera (Roth) Kützing	+	+
Cladophora sp.	+	
Ulva clathrata (Roth) C. Agardh	+	+
Ulva compressa Linnaeus	+	
Ulva fasciata Delile		+
Ulva flexuosa Wulfen	+	
Ulva intestinalis Linnaeus	+	
Ulva prolifera O.F. Müller	+	
Ulva pseudolinza (R.P.T. Koeman and Hoek) Hayden, Blomster, Maggs, P.C. Silva, M.J. Stanhope and J.R. Waaland	+	
Ulva rigida C. Agardh	+	+
Ulva sp.	+	

However, the decrease in green algae was not coupled with an increase in the abundance of species that are indicative of high water quality. Indeed, *Cystoseira mediterranea* assemblages did not show any evidence of recovery, as no significant changes were observed between 1982 and 1999. Brown algae, mainly those belonging to the order Fucales, are particularly sensitive to sewage and industrial waste (Littler and Murray 1975, Belsher and Boudouresque 1976, Kautsky et al. 1986, Tewari and Joshi 1988, Bokn et al. 1996, Díez et al. 1999, Soltan et al. 2001, Arévalo et al. 2007). Other studies have shown a rapid increase in brown algae following reductions of industrial outlets (Bokn et al. 1996, Soltan et al. 2001). However, this was not observed for *C. mediterranea* assemblages along the Catalan coast. The unchanged abundance of *C. mediterranea* assemblages between 1982 and 1999 could be explained by three different scenarios. First, green algal assemblages are substituted by *Corallina elongata*, a species that prevents *C. mediterranea* colonization through spatial exclusion. Second, the low resilience of *Cystoseira* could prevent it from returning even after one and a half decades of low disturbance. The low dispersion of *Cystoseira* zygotes (Chapman 1995) that limits new individuals to the proximity of parents (Soltan et al. 2001) could also contribute to the stagnation in the recovery of *C. mediterranea* populations. Finally, the wastewater treatment could be insufficient to allow *C. mediterranea* to recover. Thus, caution is necessary in using the natural recovery of *Cystoseira* populations to monitor the improvement in water quality, as other factors can hinder their recovery (Sales et al. 2011). Well-designed restoration projects are probably needed to enable the populations of long-lived macroalgae of the order Fucales such as *C. mediterranea* to recover (Falace et al. 2006, Susini et al. 2007, Perkol-Finkel et al. 2012).

In contrast to *C. mediterranea*, assemblages dominated by *Corallina elongata* showed significant changes.

Most of the study sites covered by green algae in 1982 shifted to being covered by *C. elongata* in 1999. The substitution of nitrophilic green algae by *C. elongata* has already been observed in Saronikos Gulf after an improvement in water quality (Tsiamis et al. 2013). In fact, several calcareous red algae have been shown to be tolerant of domestic pollution (Bellan and Bellan-Santini 1972, Kinding and Littler 1980, Díez et al. 1999, Soltan et al. 2001). However, there are also records of their decline in the surroundings of sewage outfalls (Borowitzka 1972, Brown et al. 1990). Thus, it seems that the differences in *Corallina* coverage are potentially related to the intensity and nature of the pollution (Díez et al. 1999). Moderate nutrient loads might favor the development of *Corallina*, whereas green algae become more competitive when eutrophication levels are high (Arévalo et al. 2007). Thus, we hypothesize that the increase in *C. elongata* could be the result of its physiological capacity to resist moderate water pollution combined with its ability to establish persistent populations due to its turf-like growth of erect parts and spread of basal crusts (Stewart 2008). We can conclude that the replacement of ephemeral algae by *C. elongata* is likely to be related to the decrease of nutrients loads and that *C. elongata* communities could represent an intermediate phase or a stable point in the succession from green algae-dominated environments to fucoids along an environmental quality gradient.

Changes in the total macroalgal coverage of benthic assemblages is predicted to be related to interannual variations in species abundances that are driven by different environmental factors (Ballesteros 1992, López Gappa et al. 1993) such as a higher hydrodynamism or different oceanographic conditions. In fact, in other Mediterranean areas, a decrease in the total macroalgal coverage was observed when the secondary treatment of wastewater was implemented, and thus, organic nitrogen was removed (Tsiamis et al. 2013).

Although we do not have a good explanation for the significant decrease in the number of species from 1982 to 1999, this could be related to differences in the sorting accuracy and taxonomic competence of those who processed and identified the samples, as suggested by Rindi and Guiry (2004) and Sales and Ballesteros (2010) in other studies.

Based on the ordination of sampling sites along the first DCA axis (Figure 3A) and according to other studies performed in the same region (Arévalo et al. 2007, Pinedo et al. 2007), where water quality data (mainly inorganic nutrients) were assessed, we can conclude that the first DCA axis follows a gradient from low to high ecological quality value. We have shown that several localities that were dominated by green algal cover in 1982 were devoid of such algae in 1999, after the implementation of a widespread wastewater treatment program during the 1970s, 1980s, and 1990s, which indicates an improvement in water quality. Thus, our results show that suitable management of wastewater outflows can improve the environmental status of a water body and decrease the abundance of opportunistic species indicative of eutrophication. However, we did not observe an increase in species that were unequivocally indicative of unpolluted waters (i.e., *Cystoseira*), but rather, we noted an increase in stress-resistant *Corallina* turfs that may prevent *Cystoseira* settlement. Thus, the recovery of water quality does not necessarily imply the natural restoration of *Cystoseira* stands, at least in the time period of one to two decades, strengthening the use of transplants as a feasible alternative to assist the recovery of their populations in the short to mid-term (Falace et al. 2006, Susini et al. 2007, Sales et al. 2011). The complexity of natural processes involved in the colonization and succession of macroalgal assemblages along rocky shores seems to prevent the easy and rapid restoration of mature populations and assemblages even after the implementation of effective measures to increase water quality as required by the Water Framework Directive. Thus, it is imperative to exercise caution when using only the natural restoration of *Cystoseira* and other Fucales as signs of water quality recovery.

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